SUBUNIT DISSOCIATION–ASSOCIATION OF MITOCHONDRIAL F_1-ATPase FROM YEAST

Helmut BEGUSCH and Benno HESS
Max-Planck-Institut für Ernährungsphysiologie, Rheinlanddamm 201, D-4600 Dortmund 1, FRG

Received 26 September 1979
Revised version received 4 October 1979

1. Introduction

ATPase plays a crucial role in membrane energy coupling as it is responsible for the ATP formation in biological membranes containing a respiratory or photosynthetic apparatus (reviewed in [1–6]). The complex is composed of two multisubunit parts. One portion is designated as F_0, apparently integral with the membrane. The remaining portion of the complex is the water soluble F_1-ATPase which appears to be peripheral to the membrane. It is easily and reversibly dissociated from the membrane. F_1-ATPase bears catalytic sites responsible for ATP hydrolysis and based on SDS–polyacrylamide gel electrophoresis consists of 5 non-identical subunits (α to ϵ). Yeast ATPase has a total mol. wt ~400 000 [7].

In the cold, a loss of ATP hydrolysis activity and dissociation of the enzyme into subunits has been reported [8,9]. We report here the reversible dissociation and inactivation of the enzyme in the cold, and identify the dissociating subunit.

2. Materials and methods

All chemicals used were of research grade. Cold inactivation was performed at 4°C and pH 8.0 in 5 mM Tris–HCl with 1 mM EDTA unless otherwise stated. Experiments were conducted with preparations of the F_1-ATPase prepared by the method in [7]. Reactivation was achieved by placing the undiluted enzyme solution in a 20°C bath for 3 h; aliquots were removed for assay. ATPase activity was measured spectrophotometrically by the method in [10]. Disc electrophoresis and microdisc electrophoresis were carried out by the methods in [11] and [12], respectively.

Ultracentrifugation experiments were performed in a Spinco Model E analytical ultracentrifuge and evaluated as described [13,14]. Preparative gradient polyacrylamide slab gel electrophoresis (PAGE) was performed as in [15]. After electrophoretic separation two gel stripes were cut from the gel, stained in Coomassie blue solution (0.25%, w/v, in 45% methanol, 45% water and 10% acetic acid) and destained electrophoretically in 35% methanol and 10% acetic acid. Gels with bands of the protein designated X (see below) were cut out, homogenized and dissolved in 50 ml buffer (10 mM Tris–HCl, 2 mM EDTA, 2 mM 2-mercaptoethanol (pH 8.0)). After 3 extraction periods of 4 h at room temperature the gel suspension was centrifuged (48 000 × g, 30 min, 2°C) and filtered through glasswool. Protein X was concentrated and equilibrated by Amicon ultrafiltration (PM 30 membrane filters). End-group analysis: C-terminal amino acids were identified by carboxypeptidase A digestion [16] and N-terminal amino acids by the method in [17] and by microdansylation [16,18]. 3-Phenyl-2-thiohydantoin (PTH) amino acids after Edman degradation were determined by high pressure liquid chromatography (HPLC) [19,20]. Amino acid analysis was performed in an automatic amino acid analyzer (Biotronik). For analysis, samples were hydrolyzed in 6 N HCl for 24, 48 and 72 h at 110°C. Cysteine was determined after oxidation with performic acid [21].

3. Results

3.1. *Dissociation of F_1-ATPase*

Upon incubation of F_1-ATPase (at the optimal concentration of 7.0 mg/ml) in the cold at 4°C for 10–30 min, three bands can be identified by disc electrophoresis under non-denaturing conditions (as shown in fig.1). Two of these bands had mol. wt ~400 000 and one band (protein X) mol. wt ~60 000.

In order to compare the sedimentation velocity coefficients of the native enzyme with those of the components of the dissociated F_1-ATPase, sedimentation patterns (fig.2) were produced showing one peak with 12.6 $s_{20,w}$ (native F_1-ATPase) and two peaks with $s_{20,w}$ of 10.6 and 2.6 after incubation at 4°C for 3 h. It is noteworthy that identical results were obtained in the presence of 0.4 M guanidinium chloride at 25°C indicating a similar mode of dissociation by this denaturating agent as compared to cold treatment.

3.2. *Isolation and identification of protein X*

In order to isolate the fraction containing protein X, F_1-ATPase (10 mg) was dissolved in buffer and dissociated for 5 h at 4°C. The solution was then layered on top of a preparative gradient polyacrylamide slab gel and electrophoresed under non-denaturing conditions at 25°C. Two slices of the gel were then cut off for staining. Protein X in the unstained gel was located by matching with the stained gel. The protein was extracted and concentrated by ultrafiltration.

The homogeneity of the protein was examined by disc electrophoresis and microdisc electrophoresis as shown in fig.3. In two independent electrophoretic runs mol. wt 57 000–59 000 was calculated for the isolated protein X. The isolated protein was further analyzed in the ultracentrifuge, yielding a single symmetrical peak with 2.6 $s_{20,w}$.

The C-terminal amino acid of this protein was identified as –Gly–Leu–Ala–Val–COOH by carboxypeptidase digestion. The N-terminal amino acid was

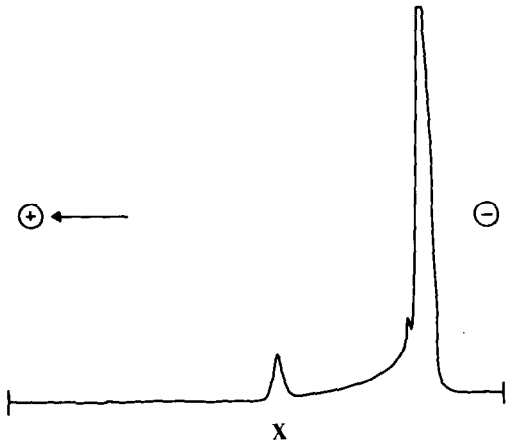

Fig.1. Microdisc electrophoresis densitogram of F_1-ATPase after 10 min cold treatment.

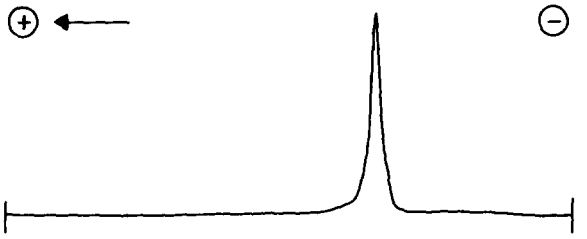

Fig.3. Microdensitogram of SDS–microdisc electrophoresis gel of protein X.

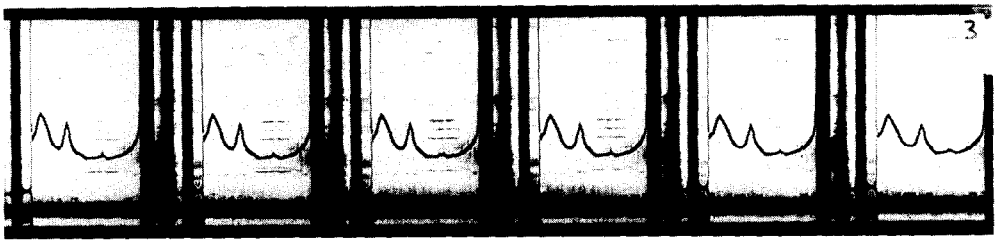

Fig.2. Sedimentation velocity analysis of cold treated F_1-ATPase. Rotor speed 60 000 rev./min at 5.7°C. Phase angle 60°, sedimentation from left to right. The photographs were taken at 4 min intervals using Schlieren optics.

Table 1
Amino acid composition of purified protein X of the mitochondrial F_1-ATPase

Amino acid[a]	Protein X
Asx	56
Thr[b]	36
Ser[b]	42
Glu	64
Pro	29
Glx	80
Ala	62
Val[c]	55
Met	19
Ile[c]	35
Leu	74
Tyr	11
Phe	16
His	11
Lys	22
Arg	21
Cys[d]	2–4
Trp[e]	–

[a] Average from 3 separate hydrolysis experiments (24, 48, 72 h)
[b] Obtained by extrapolation to $t = 0$
[c] Obtained after 72 h hydrolysis
[d] Not exactly determined
[e] Not determined

glycine. The results of amino acid analysis are shown in table 1.

3.3. *Reversibility of the dissociation of F_1-ATPase*

Dissociation of F_1-ATPase in the cold is reversible. As shown in fig.4a an analysis of the cold treated protein by gel filtration (Sephadex G-100) yielded a pattern consisting of the native and two dissociation products. When the sample was reincubated at 20°C for 3 h, then again subjected to gel filtration, most of the dissociated products disappear (fig.4b). Dissociation and reassociation is in agreement with the disappearance of the enzyme activity upon cold treatment and reappearance of its activity after incubation at room temperature (see fig.5).

4. Discussion

Our experiments show that cold-treated F_1-ATPase dissociates into two fractions, one of which we were

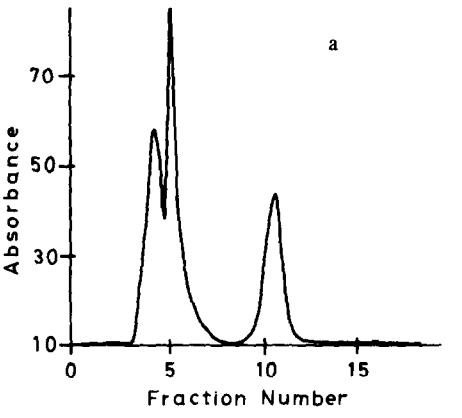

Fig.4a. Gel filtration of cold-treated F_1-ATPase after 30 min incubation, 7.0 mg protein/ml buffer at 20°C.

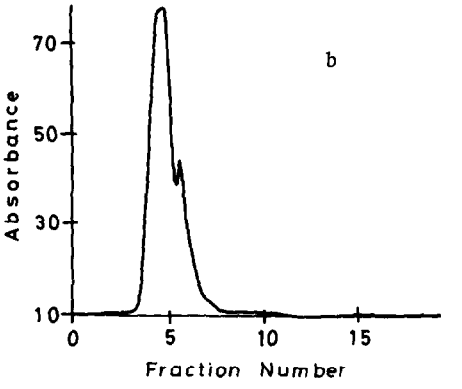

Fig.4b. Gel filtration of the reassociated F_1-ATPase sample after 30 min cold treatment under the conditions in fig.4a.

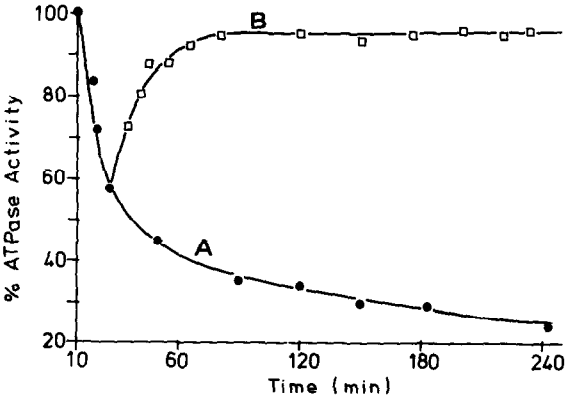

Fig.5. Plot of F_1-ATPase activity under cold treatment (A) and of reactivated F_1-ATPase (B) as a function of time. 100% = 700 units/ml.

able to isolate by preparative slab gel electrophoresis, by gel filtration and by ultracentrifugation. By the latter technique we established that F_1-ATPase was split into non-equivalent components according to the following scheme:

$$F_1 \longleftrightarrow F_1' + X$$
$$12.6\ s_{20,w} \qquad 10.6\ s_{20,w} \quad 2.6\ s_{20,w}$$

A comparison of the protein X with isolated α-subunit (H.B., B.H. unpublished) strongly suggests identity of both fractions:

(1) A mixture of protein X and the α-subunit comigrate in 0.1% SDS–gel electrophoresis yielding one single symmetrical peak by microdensitometry;
(2) The sedimentation coefficients of protein X and isolated α-subunit were closely similar;
(3) End-groups and amino acid composition of protein X and isolated α-subunit were identical;
(4) In contrast, the C-terminal amino acid of the β-subunit of yeast F_1-ATPase (H.B., B. H., unpublished) is glycine.

The reversibility of cold-induced F_1-ATPase dissociation as shown by physical as well as kinetic experiments is time-dependent. Optimal reversibility was achieved when the sample was incubated for < 30 min. This suggests that the dissociated subunit, presumably the α-subunit is only weakly bound and is essential for ATPase activity.

Our experiments support the observation of inactivation beef heart F_1-ATPase by dissociation [22]. This preparation apparently dissociates into two fragments, one of which was identified as the α- and/or β-subunit. In contrast, our data indicate that inactivation is the result of a single and reversible dissociation process by which the α-subunit is the only dissociating product, leaving the rest of F_1-ATPase (F_1') behind.

Acknowledgements

The technical assistance of M. Böhm, U. Schacknies, I. Hoffmann and M. Wieck is gratefully acknowledged.

We would like to thank Dr. H Zimmermann-Telschow and Dr M. Engelhard for their helpful discussions.

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